Effect of Flavonols on the Bacteriostatic Action of Dicoumarol

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Goth (*3*) reported that dicoumarol possessed bacteriostatic action toward certain bacteria which was not antagonized by 2-methyl-1,4-naphthoquinone (vitamin K). This would indicate that dicoumarol exerts its bacteriostatic activity through a mechanism different from that by which it induces hypoprothrombinemia and hemorrhage. In connection with some investigations in progress at this Laboratory (*2*), it was of interest to determine the effect of compounds containing the γ-pyrone structure on the antibacterial action of dicoumarol. For this purpose we have used the flavonol glycosides, rutin (*1*) and quercitrin, and the aglycone, quercetin. The effect of rutin was of especial interest, since it has pronounced physiological activity in diminishing the tendency to hemorrhage by restoring fragile capillaries to normal (*4, 5*).

The tests were made in nutrient broth (peptone, 0.5 per cent; beef extract, 0.3 per cent; and sodium chloride, 0.5 per cent) adjusted to pH 6.95. Nutrient broth solutions containing desired concentrations of dicoumarol and flavonols were dispensed in 5-ml. quantities in test tubes, sterilized by autoclaving at 15 pounds for 15 minutes, inoculated with 0.01 cc. of a 16-hour broth culture of *Staphylococcus aureus* (F.D.A. 209P), and incubated at 37° C. The antagonistic effect of the flavonols on dicoumarol was determined by using a Klett-Summerson photoelectric colorimeter to measure the density of bacterial growth in the presence of increasing quantities of the flavonols.

The results in Table 1 show that all three flavonols were capable of neutralizing the bacteriostatic action of dicoumarol. The inhibitory effect of 0.04 mg./ml. of dicoumarol was overcome by 0.05 mg./ml. of rutin and completely neutralized by 0.5 mg./ml. Higher concentrations of dicoumarol required increased amounts of rutin to show proportional antagonism. Rutin *per se* does not appear to have any effect on the growth of *Staph. aureus*.

Quercitrin was somewhat less effective than rutin as an antagonist toward dicoumarol. This may be partly due to the fact that in high concentrations quercitrin exhibits toxicity toward *Staph. aureus*. In concentrations up to 0.1 mg./ml. it showed increasing antagonism toward dicoumarol; however, above this value the toxic effect began to show up, and at 1.0 mg./ml. there was a 64 per cent inhibition in the growth of *Staph. aureus*.

Quercetin was the least effective of the three flavonols tested. It did not overcome the bacteriostatic effect of 0.08 mg./ml. of dicoumarol, and showed only partial antagonism to the lower concentrations. It exhibited considerable toxicity toward *Staph. aureus*, completely inhibiting the growth in a concentration of 0.1 mg./ml.

The antibacterial action of quercitrin is probably due to the presence of some quercetin from the hydrolysis of the rhamnoside. Investigation of the sample of quercitrin by ultraviolet absorption revealed a small quantity of quercetin. As little as 4–5 per cent of quercetin would be sufficient to produce the degree of bacteriostasis observed in this experiment.

TABLE 1

ANTAGONISTIC EFFECT OF RUTIN, QUERCITRIN, AND QUERCETIN ON THE BACTERIOSTATIC ACTIVITY OF DICOUMAROL TOWARD *Staph. aureus*

(Expressed as turbidity readings on Klett-Summerson colorimeter scale)

		Dicoumarol (mg./ml.)			
		0.0	0.02	0.04	0.08
Rutin (mg./ml.; 22 hrs. at 37°C.)	0.0	62	47	0	0
	0.01	66	50	0	0
	0.05	62	58	26	0
	0.5	62	55	59	42
Quercitrin (mg./ml.; 15 hrs. at 37°C.)	0.0	47	27	0	0
	0.05	48	48	35	0
	0.1	52	53	39	21
	0.5	44	38	30	29
	1.0	17	16	13	14
Quercetin (mg./ml.; 19 hrs. at 37°C.)	0.0	45	29	0	0
	0.01	48	42	16	0
	0.05	38	22	13	0
	0.10	0	0	0	0

This is the first time that the flavonols have been shown to possess any antibiotic action. The discovery is especially timely, considering the present intensified interest in the subject of antibiotics derived from plants. The results also suggest the possibility of using rutin or the other flavonols to antagonize the hemorrhagic action of dicoumarol *in vivo*.

References

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